TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35.U.S.C. 371

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U.S. APPLICATION NO
09/743391

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/04814	July 8, 1999	July 9, 1998

TITLE OF INVENTION
METHOD FOR PREPARING (1R,4S)-2-AZABICYCLO[2.2.1] HEPT-5-ENE-3-ONE DERIVATIVES

APPLICANT(S) FOR DO/EO/US
Christine Bernegger-Egli, C., Frank Brux, Jean Paul Roduit, Oleg Werbitzky, Yves Guggisberg

Applicant herewith submits to the United States Designated /Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An unexecuted oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [X] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
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17. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5):	
Neither international preliminary examination fee (37 CFR 1.482) Nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO (1.492(a)(3)) $1,000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO (1.492(a)(5) $860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO(1.492(a)(2)) $710.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) (1.492(a)(1)) $690.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [x] 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$ 130.00

Claims	Number Filed	Number Extra	Rate		
Total Claims	24-20=	4	X $ 18.00	$ 72.00	
Independent Claims	5-3=	2	X $ 80.00	$ 160.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				$1,492.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					
SUBTOTAL =				$1,492.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$1,492.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$1,492.00	
				Amt. refunded	$
				charged	$

a. [X] A check in the amount of $1,492.00 to cover the above fees is enclosed.

b. [ ] Please charge our Deposit Account No. 02-4377 in amount of $___ to cover the above fees. A copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4377. A copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

By Carmella L. Stephens Reg. No. 41,328

SEND ALL CORRESPONDENCE TO:

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Signature: Henry Tang

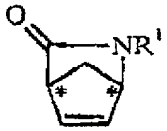
Date: January 9, 2001

29,705
Registration No.

TRANSLATION

Description

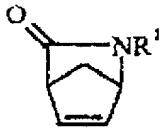
This invention involves a biotechnological method for preparing optically active compounds of the general formulas



I

II

starting with a lactam of the general formula



III

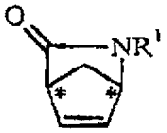
Formula I compounds, such as, for example, (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one are important intermediates for preparing (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene, which, in turn, is an important intermediate for preparing carbocyclic nucleosides, such as, for example, Carbovir (Campbell et al. J. Org. Chem. 1995, 60, 4602-4616). Formula II compounds, such as, for example, the propyl ester of (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic acid, are an important intermediate for preparing (1S,4R)-1-amino-4-(hydroxymethyl) -2-cyclopentene, which similarly can be an important intermediate for preparing carbocyclic nucleosides.

Only the chemical preparation of (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one by acylating (1R,4S)-2-azabicyclo[2.2.1]hept-5-ene-3-one (Katagiri et al., Tetrahedron Letters, 1997, 38, 1961) is known. According to this method, (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one can be obtained only from the corresponding (1R,4S)-2-azabicyclo[2.2.1]hept-5-ene-3-one as an educt. This educt is too expensive.

The problem involved in this invention was to develop a method for preparing compounds of general formula I and II, which can be prepared from easily obtainable, inexpensive starting material with good enantiomer purity.

This problem is solved with the new biotechnological method according to Claim 1.

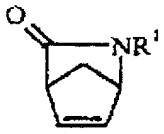
The invention's method for preparing compounds of the general formulas



I

II

wherein R^1 is acyl or acyloxy and R^2 is a hydrogen atom or C_{1-10} alkyl, takes place by means of a hydrolase in the presence of a nucleophile and in the presence of a base in a constant pH range starting with a racemic lactam of the formula



III

The starting material, the lactam of the general formula III (substrate), can be prepared, for example, according to Taylor et al. (Tet. Asymmetry. 4, 1993,1117).

C_{1-10} alkyl is linear or branched and substituted or unsubstituted. Examples of C_{1-10} alkyl are methyl, ethyl, propyl, butyl, isobutyl, t-butyl, isopropyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl and its isomers, as well as chloromethyl, bromomethyl, dichloromethyl, dibromomethyl, chloropropyl and bromobutyl.

Acyl means alkanoyl or arylcarbonyl. Alkanoyl is suitably C_{1-4} alkanoyl, which can be substituted or unsubstituted. Substituted C_{1-4} alkanoyl in the following is understood to be substituted with one or more halogen atoms. Examples of C_{1-4} alkanoyl are acetyl, propionyl, butyryl, chloroacetyl, bromoacetyl and dichloroacetyl. Arylcarbonyl is suitably benzylcarbonyl or phenylcarbonyl, substituted or unsubstituted.

Acyloxy means alkoxycarbonyl or aryloxycarbonyl. Alkoxycarbonyl is suitably C_{1-4} alkoxycarbonyl, such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl or t-butoxycarbonyl (BOC). Aryloxycarbonyl is suitably benzyloxycarbonyl or phenyloxycarbonyl.

R^1 is preferably C_{1-4} alkanoyl or C_{1-4} alkoxycarbonyl, in particular acetyl or ethoxycarbonyl.

Hydrolases that can be used are proteases or lipases, preferably proteases, such as serinproteases. Examples of serinproteases that can be used are chymotrypsins, trypsins and subtilisins (bacterial serinproteases). Subtilisins that can be used are commercial subtilisins, such as subtilisin A, subtilisin B, alcalases, ALK enzymes, bacillopeptidase A, bacillopeptidase B, bioprases, colistinases, esperases, genenase I, kazusase, maxacal, maxatases, nagarses, peptidases, protease S, protease VIII, protease XXVII, proteinases, such as the alkaline proteinase of Bacillus subtilis or Aspergillus oryzae, proteinase K from Tritirachium albumin, savinases, subtilopeptidasen, superases, and thermoases. Conducting the biotransformation by means of savinases is preferred. Suitable savinases are savinase 12 Type W™, savinase 16.OL Type EX™, savinase 32.OL Type EX™, savinase 4.OT Type W™, and savinase 8.OL™. The lipase that can be used is, for example, lipase from Candida antarctica.

If the hydrolases used are proteases, such as savinases, proteases from Bacillus subtilis, proteases from Aspergillus oryzae, proteinase K from Tritirachium albumin, the (1S,4R) enantiomer in the racemic lactam of formula III is hydrolyzed suitably into the corresponding compound of general formula II, whereby the (1R,4S) enantiomer of general formula I is

obtained. If the hydrolases used are lipases, such as lipase from Candida antarctica, the (1R,4S) enantiomer in the racemic lactam of formula III is hydrolyzed into the corresponding compound of general formula II, whereby the (1S,4R) enantiomer of general formula I is obtained.

Hydroxide ions, water or C_{1-10} alcohols can be used as the nucleophile. Suitable C_{1-10} alcohols are methanol, ethanol, propanol, isopropanol, butanol, t-butanol, isobutanol, pentanol, hexanol, heptanol, octanol, nonanol or decanol. If the nucleophile used is a C_{1-10} alcohol, the corresponding ester of general formula II (R^2 = C_{1-10} alkyl) is formed, as the expert knows. If water is used as the nucleophile, obviously, the corresponding acid of general formula II (R^2 = H) is formed.

Depending on the hydrolase and the substrate (formula III lactam), the biotransformation is conducted suitably between pH 5 and 12, preferably between pH 6 and 8. In the invention, for a given hydrolase and a given substrate, the pH value is maintained constant in the presence of a base. The pH value is suitably maintained constant at +/- 0.5 pH units by addition of a base. If, for example, the substrate is racemic 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (R^1 = acetyl) and savinase is used as the hydrolase, the pH value is held constant at preferably between pH 7.0 and pH 7.5.

An inorganic or organic base can be used as the base. For example, KOH and NaOH are suitable as inorganic bases. A suitable organic base can be, for example, triethanolamine dissolved in an organic solvent. If one of the aforesaid alcohols is used as the nucleophile, the corresponding alcoholate can serve as the base.

The conversion temperature can be in the range of 10 to 60° C, preferably 15 to 40° C.

The biotransformation is suitably conducted in water, a buffer solution, a C_{1-10} alcohol or in a mixture of these with an aprotic organic solvent. Suitable aprotic organic solvents are, for example, ether and aromatic hydrocarbons. Tetrahydrofuran, dioxane or t-methylbutyl ether can be used as the ether. Toluene and benzene are suitable aromatic hydrocarbons. The buffer solutions used can be, for example, low molarity, such as 10 – 100 mM sodium or potassium phosphate buffer, hepes buffer. The C_{1-10} alcohols used can be those previously described.

The biotransformation can also be conducted so that the lactam of general formula III serves as solvent. Then the biotransformation is suitably conducted in the presence of half of the stoichiometric quantities of water or the corresponding alcohol.

Depending on the solvent, the biotransformation can be conducted in a two-phase or one-phase system. The biotransformation is advisedly conducted in a one-phase system.

After a usual conversion time of a few hours depending on the selected starting material, the desired optically active compounds of general formulas I and II are obtained in outstanding yields and enantiomer purity. The preferred starting materials are racemic 2-acetyl-2-azabicyclo-[2.2.1]hept-5-ene-3-one (R^1 = acetyl) and racemic 2-ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one. The preferred products of the compounds of general formula I are (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (R^1 = acetyl) and (1R,4S)-2-ethoxy-carbonyl-2-azabicyclo-[2.2.1]hept-5-ene-3-one (R^1 = ethoxycarbonyl). The preferred compounds of general formula II are (1S,4R)-1-acetylamino-2-cyclopentene-4-carboxylic acid (R^1 = acetyl, R^2 = H), (1S,4R)-1-

ethoxycarbonyl-2-cyclopentene-4-carboxylic acid (R^1 = ethoxycarbonyl, R^2 = H), (1S,4R)-1-acetylamino-2-cyclopentene-4-carboxylic acid methyl ester (R^1 = acetyl, $R^2 = CH_3$), (1S,4R)-1-acetylamino-2-cyclopentene-4-carboxylic acid butyl ester (R^1 = acetyl, $R^2 = C_4H_9$), (1S,4R) acetylamino-2-cyclopentene-4-carboxylic acid ethyl ester (R^1 = acetyl, $R^2 = C_2H_5$ and (1S,4R)-1-acetylamino-2-cyclopentene-4-carboxylic acid propyl ester (R^1 = acetyl, $R^2 = C_3H_7$). The (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic acid C_{2-10} alkyl esters, except the (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic acid ethyl ester, preferably the (1S,4R)-acetylamino-2-cyclopentene-4-carboxylic acid ethyl ester and the (1S,4R)-acetylamino-2-cyclopentene-4-carboxylic acid propyl ester of general formula II are not described in the literature and consequently are similarly part of the invention.

Another part of the invention is the subsequent reaction, the reduction of compounds of general formula I to an optically active 1-amino-4-(hydroxymethyl)-2-cyclopentene derivative, in particular to a (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene derivative of the general formula

IV

wherein R^1 is as already named.

The reduction is suitably conducted with binary or complex metal hydrides of the boron or aluminum group, such as alkali metal borohydrides, alkaline earth metal borohydrides, alkali metal aluminum hydrides, alkaline earth metal aluminum hydrides.

The binary alkali metal borohydrides or alkaline earth metal borohydrides used can be $NaBH_4$, $LiBH_4$, KBH_4, $NaAlH_4$, $LiAlH_4$, $KAlH_4$, $Mg(BH_4)_2$, $Ca(BH_4)_2$, $Mg(AlH_4)_2$, $Ca(AlH_4)_2$. Complex metal hydrides of the boron or aluminum group can have the general formula $M^1M^2H_nL_m$, wherein n is a whole number from 1 to 4, m is a whole number from 4 to 4 minus the corresponding number n, M^1 is an alkali metal atom, M^2 is boron or aluminum, and L is C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkoxy, CN or an amine, or the complex metal hydrides can have the general formula $M^2H_OL_p$, wherein M^2 is as already named, O is a whole number from 0 to 3, and p is a whole number from 3 to 3 minus the corresponding number p. The $M^1M^2H_nL_m$ used can be $LiBH(C_2H_5)_3$, $LiBH_x(OCH_3)_{4-1}$, wherein x is a whole number from 1 to 3, $LiAlH(OC(CH_3)_3)_3$, $NaAlH_2(OC_2H_4OCH_3)_2$, $NaAlH_2(C_2H_5)_2$ or $NaBH_3CN$. The reduction is conducted preferably with a metal borohydride, such as sodium borohydride.

The metal hydrides are used suitably in a molar ratio of 0.5 to 1 per mole of compound of general formula I.

The reduction is conducted suitably under an inert gas atmosphere, such as, for example, under an argon or nitrogen atmosphere.

The reduction can be conducted at a temperature of –10 to 30° C, preferably 0 to 10° C.

Secondary or tertiary alcohols are suitable as solvents for the reduction. 2-Butanol can be used, for example, as the secondary alcohol, and t-amyl alcohol, for example, as the tertiary alcohol. A secondary alcohol is preferred.

The subsequent conversion, the hydrolysis of the optically active 1-amino-4-(hydroxymethyl)-2-cyclopentene derivatives of formula IV to the corresponding optically active 1-amino-4-(hydroxymethyl)-2-cyclopentenes or their salts of the formula

V

with an alkaline earth metal hydroxide, alkali metal hydroxide or with a mineral acid is similarly part of the invention. In particular, the (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene derivative is hydrolyzed to (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene.

Lithium, sodium or potassium hydroxide is suitable as the alkali metal hydroxide. Barium hydroxide, for example, can be used as the alkaline earth metal hydroxide. The hydrohalide acids such as, for example, hydrochloric acid or hydrobromic acid are suitable as the mineral acids.

The hydrolysis is suitably conducted at a temperature of 50 to 120° C, preferably 90 to 100° C.

Suitable salts of the (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene (formula V) are its hydrohalide salts, such as hydrochlorides or hydrobromides.

Examples

Example 1
Preparation of (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene from racemic (±)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one

1.1 Preparation of (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one

1.1.1 By savinase in a mixture of sodium phosphate buffer and tetrahydrofuran

5 ml tetrahydrofuran were mixed with 3.84 ml 20 mM sodium phosphate buffer pH 7 and 1.16 ml savinase 16.0 L type EX, Novo Nordisk (16 KNPU/g, kilo novo protease units). 417 µl (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (330 mmoles/l) were added. The reaction was mixed with a magnetic stirrer and maintained at 30° C by a water bath. The pH value was maintained constant at pH 7 by a pH automatic control system with 1 M NaOH. Samples were taken periodically and analyzed by HPLC for content and enantiomer excess (Chiralpak AD, Daicel Chemical Ltd.(0.46 x 25 cm) isocratically at room temperature, flow 1 ml/min with n-heptane (ethanol 2%, detection at 215 nm)). A total of 1.25 ml NaOH were used. After 120 minutes, 50% of the quantity of (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one used was hydrolyzed to the corresponding acid. The content was determined with achiral GC. The analysis was conducted with gas chromatography as follows: capillary column: HP – 5 (5%

phenylmethylsiloxane), temperature gradient 100° C – 260 ° C; the samples were diluted in tetrahydrofuran 1:1 for the analysis.

1.1.2 In Water

1.1.2.1 60 ml H_2O and 35 ml savinase were added to 419.25 ml (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one as in example 1.1.1. The pH value was maintained at 7.5 with 7.5N NaOH. The 1 l Applikon fermenter was stirred at 400 rpm. Samples were taken periodically and analyzed as in Example 1.1.1. An ee [errors excepted] value of 99% was measured after 45 hours.

The charge was filtered by suction through a Whatman GF/F filter, and the filter cake was washed twice with 100 ml and once with 50 ml butyl acetate. The aqueous phase was shaken twice with 200 ml butyl acetate. The organic phase was concentrated in a Rotovap. 144.8 g of product were obtained with an ee value of >98%, which corresponded to a yield of 31% relative to the racemate. The analysis was conducted as in Example 1.1.1.

1.1.2.2 60 ml H_2O and 35 ml savinase were added to 486.3 g (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) as in Example 1.1.1. The pH value was maintained at 7.5 with 7.5N NaOH. The 1 l Applikon fermenter was stirred at 400 rpm. Samples were taken periodically and analyzed as in Example 1.1.1. An ee value of >98% was measured after 45 hours. A total of 174.6 ml 7.5N NaOH were used.

The charge was filtered by suction through a Whatman GF/F filter, and the filter cake was washed twice with 100 ml and once with 50 ml butyl acetate. The aqueous phase was shaken twice with 200 ml butyl acetate. The organic phase was concentrated in a Rotovap. 144.8 g of product were obtained with an ee value of >98% (content: 92.5%), which corresponded to a yield of 29% relative to the racemate. The analysis was conducted as in Example 1.1.1.

1.1.3 In Methanol

72.3 ml (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) were mixed with 8.3 ml savinase as in Example 1.1.1 and 19.4 ml methanol. The reaction mixture was stirred at 30 ° C for 24 hours. The pH remained constant at 7.2. Samples were taken as in Example 1.1.1. After 25 hours, an ee value of >98% was reached, and the reaction was ended. The analytical yield was 42% relative to the racemate. The corresponding methyl ester ($R^2 = CH_3$) of the compound of general formula II, which was proven by GC-MS, was formed in stoichiometric quantities. The analysis was conducted as in Example 1.1.1.

1.1.4 In Butanol

72.3 ml (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) were mixed with 76.86 ml 1-butanol and 8.3 ml savinase as in Example 1.1.1. The reaction mixture was measured during a time period of 22 hours. After this time, an ee value of >98% was determined with HPLC. The pH value was maintained constant at pH 7.5 with 4 N NaOH. Samples were taken as in Example 1.1.1. The reaction temperature was 30° C. The formation of a free acid (R^2 = H) of the compound of general formula II was proven with HPLC, and the formation of the butyl ester (R^1 = C_4H_9) of the compound of general formula II was proven by GC-MS. An analytical yield of 34.8% relative to the racemate was achieved.

1.1.5 By Savinase in 1-Propanol

1.1.5.1 242 g (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one, 168.6 ml 1-propanol and 28 ml savinase as in Example 1.1.1 were incubated at 30° C and at a pH of 7.0 (adjusted with 4N NaOH). Samples were taken as in Example 1.1.1. After 24 hours, the ee value of the product was determined at % ee = 99%. The analytical yield was 47%. 100 ml toluene were added to this mixture (459 ml), and the propanol was evaporated under reduced pressure. The solution was extracted twice with toluene (250 ml) and water (100 ml). The toluene was evaporated and the product distilled. 113.9 g (0.69 mole) of product (purity 93%, ee = 99%) corresponding to a yield of 45.7% relative to the racemate were obtained. The analysis was conducted as in Example 1.1.1.

1.1.5.2 208 g (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%), 168.6 ml 1-propanol and 23 ml savinase as in Example 1.1.1 were incubated at 30° C and at a pH of 7.2 (adjusted with 4N NaOH) in an Erlenmeyer flask. After 30 hours, the ee value of the product was determined at % ee = >98%. 100 ml toluene were added to this mixture (459 ml), and the propanol was evaporated under reduced pressure. The solution was extracted twice with toluene (250 ml) and water (100 ml). The toluene was evaporated and the product distilled (12 mbar, 85 - 95° C). 79.13 g (0.69 mole) of product (purity 93%, ee >98%) corresponding to a yield of 37% relative to the racemate were obtained. The analysis was conducted as in Example 1.1.1.

1.1.5.3 2.77 kg (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.8%), 1.35 l of 1-propanol and 355.5 ml savinase as in Example 1.1.1 were reacted at 40° C and at a pH of 7.4 (set by the addition of 92 g 4N NaOH) in a 5 l stirred reactor. The pH value was maintained constant at pH = 7.4 by the addition of 4 N NaOH. After 14 hours, the reaction mixture was cooled to room temperature, and the pH of the solution was adjusted to pH = 7.0 by the addition of 20% sulfuric acid. After the addition of 1.15 l toluene, the phases were separated, and the organic phase was vacuum-distilled (product fraction at T = 72 - 76° C, p = 1 mbar). 1.07 kg of product (content 91%, ee = 98.4%)were obtained, corresponding to a yield of 36.5%. The

product content was determined by GC in an Optima 5 column (Macherey-Nagel, Germany). The ee of the product was determined by GC on a chiral LipodexE column (Macherey-Nagel, Germany).

1.1.5.4 Isolation of (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic Acid Propyl Ester

345 g of the still residue from Example 1.1.5.3 were mixed with 250 ml ethyl acetate and 1 l hexane, and the mixture was heated to reflux. Two phases formed. The upper phase was separated and cooled slowly to 0° C. The precipitate was filtered off and vacuum-dried at 40° C. 85.6 g of a colorless product were obtained.

The ee value was determined by GC on a chiral Hydrodex-beta-PM column (Macherey-Nagel, Germany), ee = 93%.

α [illegible] $_c$(c = 1. MeOH) = 79.3°

^{1}H-NMR($CDCl_3$) 5.91 (br, 1H)
5.89 (s, 2H)
5.07 (m, 1H)
4.07 (t, 2H)
3.50 (m, 1H)
2.46 (m, 1H)
1.96 (s, 3H)
1.90 (m, 1H)
1.67 (m, 2H)
0.96 (t, 3H)

1.1.6 By Protease from Bacillus Subtilis in Phosphate Buffer

1.1.6.1 25 mg Bacillus subtillis protease (Fluka 82490), 0.45 ml phosphate buffer (100 mM, pH 7.5), 0.5 ml n-propanol and 0.05 ml (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 6 hours, all of the (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was converted. 1.5 hours after incubation, the enantiomer excess for (-)-2-acetyl-2-azabicyclo[2.2.1]-hept-5-ene-3-one was >98%. The unisolated yield was 12% relative to the racemate. Content and enantiomer excess were determined as in Example 1.1.1.

1.1.6.2 125 mg Bacillus subtillis protease (Fluka 82490), 2.25 ml phosphate buffer (100 mM, pH 7.5), 2.5 ml n-propanol and 0.25 ml (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 6 hours, 90% of the (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-

ene-3-one was converted. 1.5 hours after incubation, the enantiomer excess for (-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was >98%. The unisolated yield was 12% relative to the racemate.

1.1.7 By Protease from Aspergillus Oryzae in Phosphate Buffer

1.1.7.1 25 mg Aspergillus oryzae (Sigma P-4032, 3.5 units/mg), 0.45 ml phosphate buffer (100 mM, pH 7.5), 0.5 ml n-propanol and 0.05 ml (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 0.5 hour, all of the (+)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was converted. The enantiomer excess for (-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was >98%. The analytical yield relative to (-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was >40% relative to the racemate. Content and enantiomer excess were determined as in Example 1.1.1.

1.1.7.2 125 mg Aspergillus oryzae (Sigma P-4032, 3.5 units/mg), 2.25 ml phosphate buffer (100 mM, pH 7.5), 2.5 ml n-propanol and 0.25 ml (+/-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 0.5 hour, all of the (+) 2-acetyl-2-azabicyclo-[2.2.1]hept-5-ene-3-one was converted. The enantiomer excess for (-) 2-acetyl-2-azabicyclo-[2.2.1]hept-5-ene-3-one was >98%. The analytical yield relative to (-) 2-acetyl-2-azabicyclo-[2.2.1]hept-5-ene-3-one was >40% relative to the racemate.

1.1.8 By Proteinase K from Tritirachium Albumin in Phosphate Buffer

1.1.8.1 25 mg proteinase K (Sigma P-8044, 1 - 7 units/mg), 0.45 ml phosphate buffer (100 mM, pH 7.5), 0.5 ml n-propanol and 0.05 ml (+/-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 0.5 hour, all of the (+) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was converted. The enantiomer excess for (-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was >98%. The analytical yield relative to (-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was 40% relative to the racemate. Content and enantiomer excess were determined as in Example 1.1.1.

1.1.8.2 125 mg proteinase K (Sigma P-8044, 1 - 7 units/mg), 2.25 ml phosphate buffer (100 mM, pH 7.5), 2.5 ml n-propanol and 0.25 ml (+/-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 0.5 hour, all of the (+) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was converted. The enantiomer excess for (-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-

3-one was >98%. The analytical yield relative to (-) 2-acetyl-2-azabicyclo[2.2.1]- hept-5-ene-3-one was 30% relative to the racemate.

1.2 Preparation of (1S,4R)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (by Lipase from Candida Antarctica)

1.2.1 25 mg SP525 (Novo Nordisk), 0.45 ml phosphate buffer (100 mM, pH 7.5), 0.5 ml n-propanol and 0.05 ml (+/-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 0.5 hour, all of the (-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was converted. The enantiomer excess for (-)(1S,4R)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was >98%. The analytical yield relative to (+) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was 12% racemate. Content and enantiomer excess were determined as described in Example 1.

1.2.2 250 mg SP525 (Novo Nordisk), 2.25 ml phosphate buffer (100 mM, pH 7.5), 2.5 ml n-propanol and 2.25 ml (+/-) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (content: 95.4%) were incubated at 30° C (+/- 2° C). The pH value was maintained at 7.5 by the manual addition of 1 N NaOH, whereby fluctuations of +/- 0.5 pH were attained. Samples were taken as in Example 1.1.1. After 16 hours, all of the (+/-)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was converted. The enantiomer excess for (+)(1S,4R)-2-acetyl-2-azabicyclo[2.2.1]- hept-5-ene-3-one was >98%. The analytical yield relative to (+) 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one was 12% relative to the racemate.

1.3. Reduction of (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one to (1R,4S)-2-Acetyl-1-amino-4-(hydroxymethyl)-2-cyclopentene

1.3.1 8 g $NaBH_4$ were added portionwise to a solution of (1R,4S)-2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (50 g, 0.33 mole), 40 ml water and 240 ml 2-butanol, and the temperature was maintained below 5° C. After 1 hour, the reaction was stopped, and the reaction mixture was adjusted to pH 2.0 with concentrated HCl. The reaction temperature was maintained below 10° C. The pH value was adjusted to pH 9.0 with 30% NaOH. Sodium metaborate was filtered off and the aqueous phase was extracted three times with 2-butanol. After evaporation of the 2-butanol, 49.3 g of product (0.28 mole) were obtained, corresponding to a yield of 88%.

1.3.2 287.4 g (-)-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one (100% ⇒ -255 ml, 97%; 1.9 moles) were dissolved in 380 ml water and 1217 ml 2-butanol. The solution was cooled to 0 to -2° C. 45 g $NaBH_4$ (1.188 moles, 1.25 eq.) were suspended in 304 ml fresh 2-butanol in another stirring device. The $NaBH_4$ suspension was added to the solution during 1 – 2 hours. The reaction was exothermic, and the temperature was not allowed to exceed 5° C. The temperature had to be at 0° C before a portion was added. The reaction was followed by DC (thin-layer

chromatography) (hexane/etrol/MeOH: 5/5/1) The reaction was allowed to continue for 1 to 2 hours after the addition. When the conversion was complete (educt concentration had to be at <1.0%), the pH was adjusted to 2 with ca. 135 g concentrated hydrochloric acid. The temperature was maintained below 10° C. The pH was then adjusted immediately to 9 with ca. 85 ml 30% sodium hydroxide solution. The precipitated salts were filtered and washed with 127 ml fresh 2-butanol. The filtrate and the "2-butanol wash" were combined, and the phases separated. The aqueous phase was extracted twice with 380 ml fresh 2-butanol each time. The 2-butanol phases were combined. Ca. 2450 g of a 10% solution of the product, (1R,4S)-2-acetyl-1-amino-4-(hydroxymethyl)-2-cyclopentene, were obtained in 2-butanol. This corresponded to ca. 250 g of 100% product, (1R,4S)-2-acetyl-1-amino-4-(hydroxymethyl)-2-cyclopentene, corresponding to a yield of 85%.

1.4 Hydrolysis of (1R,4S)-2-Acetyl-1-amino-4-(hydroxymethyl)-2-cyclopentene to (1R,4S)-1-Amino-4-(hydroxymethyl)-2-cyclopentene

1.4.1 30% NaOH (45 g) was added to 49.3 g (0.28 mole) (1R,4S)-2-acetyl-1-amino-4-(hydroxymethyl)-2-cyclopentene, and the suspension was heated to 100° C. After 3.5 hours, the solution was cooled to 0° C and then adjusted to pH = 1.0 with concentrated HCl. Water was evaporated and NaCl filtered off. Pentanol (2 ml per g of residue) and acetone (6 ml per gram of residue) were added. The resulting precipitate was filtered and washed with 20 ml acetone. 37.5 g (0.24 mole) of product were obtained as the hydrochloride salt having an ee = 99%, corresponding to a yield of 86%.

1.4.2 85.4 g (-)-2-acetyl-1-amino-4-(hydroxymethyl)-2-cyclopentene 100% (0.55 mole) was prepared as a 10% solution in 2-butanol. It was distilled until the distillate ceased. Then 110.0 g of a 30% sodium hydroxide solution (⇒ 33.0 g NaOH 100%; 0.825 mole, 1.5 eq.) and 65 g water were added. The remaining 2-butanol was removed (with ca. 10 g water) by azeotropic distillation. The solution was heated at reflux (100 - 110° C) for 4 – 5 hours. The reaction was followed by GC. When the conversion was complete, the reaction was cooled to 50° C, and 154 ml 2-butanol (124.3 g) were added. The phases were separated at 50° C, and the aqueous phase was once again extracted with 154 ml 2-butanol (124.3 g) at 50° C (15 minutes stirring, phases separate). The aqueous phase (ca. 165 g) was discarded. The organic phases were combined, and ca. 22 g hydrogen chloride were added at 20 – 40° C to make pH 1. Some salts precipitated during the acidification. These salts were filtered off at 20° C, and the filtrate was distilled under standard pressure until 220 ml distillate (ca. 180 g) were collected (boiling temperature ca. 91 – 92 ° C). At ca. 70° C, 176 ml acetone (139.0 g) were added. The suspension was stirred at reflux for 15 – 30 minutes and then cooled to -5° C. After 1 hour at this temperature, the suspension was filtered off by suction, and the filter cake was washed with 154 ml acetone. 70 g of (-) of 100% product were obtained, corresponding to a yield of 85%.

Content: 99.0% (Tit., wt %)
NaCl: 0.5 to 1.0%

Example 2
Preparation of (1R,4S)-2-Ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one

2.1 Preparation of racemic (±)-2-Ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one

109.13 g racemic 2-ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one were mixed with 182.1 g triethylamine, 6.11 g 4-dimethylaminopyridine and 500 ml acetonitrile. The reaction was heated to 50° C. Then, 195.3 g ethyl chloroformate, dissolved in 150 ml acetonitrile, were added portionwise. The temperature was maintained below 55° C. After the reaction ended, the solution was cooled to 20° C. The salts were filtered off and washed with acetonitrile. The filtrate was concentrated at 60° C and 20 mbar, and then mixed with 1500 ml toluene. Three extractions followed: with 250 ml water, pH 8, with 250 ml acetic acid (1%) and with 250 ml saturated NaCl solution. The organic phase was dried with $MgSO_4$ and concentrated at 80° C/20 mbar. 167.4 g of a brown oil were obtained. The content by GC was 96% (±)-2-ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one, which was purified by vacuum distillation, $b.p._{0.01}$ = 76.5 ° C, content (GC): 99.5%, yield: 156.6 g (88.5%).

^{1}H-NMR ($CDCl_3$)	1.33 (t, 3H),
	2.19 (d, 1H),
	2.38 (d, 1H),
	3.43 (s, 1H),
	4.26 (m, 2H).
	5.04 (s, 1H),
	6.68 (m, 1H),
	6.92 (m, 1H).

2.2 Preparation of (-)-2-Ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one

The product was prepared as in Example 2.1, starting from (-)-2-ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one.

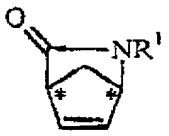
2.3 Preparation of (1R,4S)-2-Ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one

500 µl savinase as in Example 1.1.1, 5 ml n-propanol, 4.5 ml (50 mM phosphate buffer pH 8, /tetrahydrofuran 1/1) and 250 µl (+/-) ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one were incubated at 40° C at pH 8. The pH value was maintained at 8 by the manual addition of 1 N NaOH. Samples were taken as in Example 1.1.1. After 4.5 hours, an ee value of

>98% was measured for the (-) enantiomer, which was proven by the prepared standard (Example 2.2). The unisolated analytical yield relative to the racemate was 46%.

Patent Claims

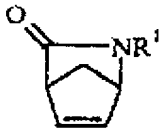
1. Method for preparing optically active compounds of the general formulas



I

II

wherein R^1 is acyl or acyloxy and R^2 is a hydrogen atom or C_{1-10} alkyl,
wherein a racemic lactam of the general formula



III

is converted by means of a hydrolase in the presence of a nucleophile and in the presence of a base in a constant pH range.

2. Method according to Claim 1, characterized in that a protease or lipase is used as the hydrolase.

3. Method according to Claim 2, characterized in that a serinprotease is used as the protease.

4. Method according to Claim 3, characterized in that a subtilisin is used as the serinprotease.

5. Method according to at least one of Claims 1 to 4, characterized in that 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-one or 2-ethoxycarbonyl-2-azabicyclo[2.2.1]hept-5-ene-3-one is used as the racemic lactam of general formula III.

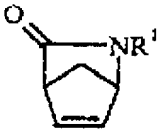
6. Method according to at least one of Claims 1 to 5, characterized in that the conversion is conducted in water, a buffer solution, a C_{1-10} alcohol or in a mixture of these with an aprotic organic solvent.

7. Method according to at least one of Claims 1 to 6, characterized in that the reaction is conducted at a temperature of 10 to 60° C.

8. Method for the preparation of optically active 1-amino-4-(hydroxymethyl)-2-cyclopentene derivatives of the general formula

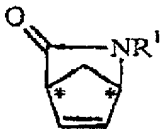
IV

wherein R^1 is the same as in Claim 1, characterized in that a lactam of the general formula



III

wherein R^1 is the same as in Claim 1, is converted by means of a hydrolase in the presence of a nucleophile and in the presence of a base in a constant pH range into the compound of the general formula



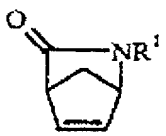
I

and this is reduced to the compound of general formula IV.

9. Method for the preparation of (1R,4S)-1-amino-4-(hydroxymethyl)-2-cyclopentene of the formula

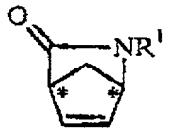
V

or its salts, characterized in that a lactam of the general formula



III

wherein R^1 is the same as in Claim 1, is converted by means of a hydrolase in the presence of a nucleophile and in the presence of a base in a constant pH range into the compound of the general formula



I

wherein R^1 is the same as in Claim 1, this is then reduced to the compound of the general formula

IV

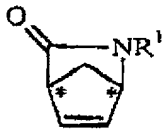
wherein R^1 is the same, and this is then hydrolyzed to the compound of formula V.

10. (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic acid $C_{2\text{-}10}$ alkyl esters, excluding (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic acid ethyl ester.

11. (1S,4R)-acetylamino-2-cyclopentene-1-carboxylic acid ethyl ester or propyl ester.

Summary

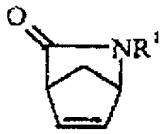
A biotechnological method is described for preparing compounds of the general formulas



I

II

wherein R^1 is acyl or acyloxy and R^2 is a hydrogen atom or C_{1-10} alkyl, comprising the conversion of a lactam of the general formula



III

by means of a hydrolase in the presence of a nucleophile and in the presence of a base in a constant pH range.

Also described is the subsequent conversion of the compound of general formula I into the optically active 1-amino-4-(hydroxymethyl)-2-cyclopentene of the formula

V

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"METHOD FOR PREPARING (1R,4S)-2-AZABICYCLO[2.2.1] HEPT-5-ENE-3-ONE DERIVATIVES"

This declaration is of the following type:

[] original
[] design
[X] national stage of PCT.
[] divisional
[] continuation
[] continuation-in-part (C-I-P)

The specification of which: *(complete (a), (b), or (c))*

(a) [ ] is attached hereto.
(b) [X] was filed on January 9, 2001 as Application Serial No. (to be assigned).
(c) [X] was described and claimed in PCT International Application No. PCT/EP99/04814 filed on July 8, 1999 and was amended on *(if applicable).*

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

[ ] In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

(d) [ ] no such applications have been filed.
(e) [X] such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
			[ ] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]
ALL FOREIGN APPLICATION[S], IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
EP 98112719.4	July 9, 1998		[X] YES NO [ ]
EP 98123949.4	December 17, 1998		[X] YES NO [ ]
			[ ] YES NO [ ]

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
PCT/EP99/04814	July 8, 1999	Pending

Power of Attorney

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge

that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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DATE 23.03.2001	SIGNATURE OF INVENTOR			

Check proper box(es) for any added page(s) forming a part of this declaration

[ ] Signature for ninth and subsequent joint inventors. Number of pages added _____.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added _____.

[ ] Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47. Number of pages added _____.